App. No. 09/073,596 Reply to Office Action of June 9, 2009 Page 2 of 17

9192876336

RECEIVED CENTRAL FAX CENTER DEC 0 8 2009

## Amendments to Claims:

This listing of claims will replace all prior versions and listings in the application:

## **Listing of Claims:**

- 1-98. (Canceled)
- 99. (Previously Presented) The pharmaceutical composition according to claim 116, wherein the dendritic cells express an amount of the modified antigen to provide between about 1 to 100 micrograms of the modified antigen in said pharmaceutical composition.
  - 100. (Canceled)
- 101. (Currently Amended) An in vitro composition comprising mature dendritic cells expressing modified antigen and derived from an in vitro culture of an enriched and expanded population of proliferating dendritic cell precursors by a method comprising:

providing a tissue source comprising dendritic cell precursors;

treating the tissue source comprising dendritic cell precursors to increase the proportion of dendritic cell precursors;

treating the tissue source to obtain a population of cells suitable for culture in vitro;

culturing the tissue source on a substrate in a culture medium comprising GM-CSF to obtain cell-aggregates comprising proliferating dendritic cell precursors nonadherent cells and cell clusters;

subculturing the cell aggregates at least one time to enrich the proportion of the nonadherent cells and cell clusters to produce cell aggregates comprising proliferating dendritic cell precursors;

serially subculturing the cell aggregates one or more times to enrich the proportion of dendritic cell precursors; and

continuing to culture the dendritic cell precursors for a period of time to allow them to mature into mature dendritic cells:

wherein the dendritic cells are cultured culturing the dendritic cells in vitro in the presence of an antigen for a time sufficient to allow the antigen to bind to

App. No. 09/073,596 Reply to Office Action of June 9, 2009 Page 3 of 17

the dendritic cells and, wherein the dendritic cells process the antigen to produce a modified antigen which is expressed by the dendritic cells.

102-103. (Canceled)

- 104. (Previously Presented) The composition according to claim 101, wherein the tissue source is blood.
- 105. (Previously Presented) The composition according to claim 101, wherein the tissue source is bone marrow.
- 106. (Previously Presented) The composition according to claim 101, wherein GM-CSF is present in the culture medium at a concentration of about 1-1000 U/ml.
- 107. (Previously Presented) The composition according to claim 104, wherein the concentration of GM-CSF in the culture medium is about 30-100 U/ml.
- 108. (Previously Presented) The composition according to claim 105, wherein the concentration of GM-CSF in the culture medium is about 500-1000 U/ml.
- 109. (Previously Presented) The composition according to claim 101, wherein the cell aggregates are blood derived and are subcultured from about one to five times.
- 110. (Previously Presented) The composition according to claim 101, wherein the cell aggregates are subcultured one to five times.
- 111. (Previously Presented) The composition according to claim 101, wherein the culture medium is selected from the group consisting of RPMI 1640, DMEM and  $\alpha$ -MEM, and wherein the culture medium is supplemented with scrum.
- 112. (Previously Presented) The composition according to claim 104, wherein the tissue source is treated to remove red blood cells.
- 113. (Previously Presented) The composition according to claim 105, wherein the tissue source is treated to remove B cells and granulocytes.

114-115. (Canceled)

App. No. 09/073,596
Reply to Office Action of June 9, 2009
Page 4 of 17

116. (Previously Presented) A pharmaceutical composition comprising a therapeutically effective amount of the composition according to claim 101.

117-119. (Canceled)

- expressing a modified antigen and derived from an *in vitro* culture of a population of enriched and expanded proliferating dendritic cell precursor cells, wherein said dendritic cells are contacted *in vitro* with antigen in the presence of GM CSF for a sufficient time to allow the antigen to bind to the dendritic cells, by a method comprising culturing dendritic cell precursor cells in a culture medium comprising GM-CSF at a concentration sufficient to promote the survival and proliferation of dendritic cell precursors; serially subculturing the proliferating dendritic cell precursors at intervals which provide for the continued proliferation of said dendritic cell precursors; and exposing the cells to antigen *in vitro*, wherein the dendritic cells process the antigen to produce a modified antigen which is expressed by the dendritic cells.
  - 121-141. (Cancelled)
- 142. (Previously Presented) The composition according to claim 101, wherein the dendritic cell precursors are human.
- 143. (Currently Amended) The composition of dendritic cell procursors according to claim 142, wherein the dendritic cell procursors are obtained from blood.
- 144. (Currently Amended) The composition of dendritic cell precursors according to claim 142, wherein the dendritic cell precursors are obtained from bone marrow.
- 145. (New) An *in vitro* composition comprising antigen-activated dendritic cells expressing modified antigens and derived from an *in vitro* culture of proliferating dendritic cell precursors by a method comprising:
  - a) providing a tissue source comprising dendritic cell precursors;
  - b) treating the tissue source to obtain a population of cells suitable for culture in vitro;
  - c) culturing the tissue source on a substrate in a culture medium comprising GM-CSF to obtain nonadherent cells and cell clusters;

App. No. 09/073,596 Reply to Office Action of June 9, 2009 Page 5 of 17

- d) subculturing the nonadherent cells and cell clusters to produce cell aggregates comprising proliferating dendritic cell precursors;
- e) serially subculturing the cell aggregates one or more time to enrich the proportion of dendritic cell precursors;
- f) continuing to culture the dendritic cell precursors for a period of time sufficient to allow them to mature into mature dendritic cells; and
- g) pulsing the dendritic cells with an antigen, wherein the dendritic cells process the antigen to produce a modified antigen which is expressed by the dendritic cells.